Supplemental Methods and Results **MS ID#: JLR/2012/031021**

Plasma plant sterols serve as poor markers of cholesterol absorption in man

Bioavailability of the oral D_7-cholesterol tracer

Rationale

To study whether the slightly lower than expected FCA rates in our study were due to lower bioavailability of the oral D_7-cholesterol tracer, caused by the method of administration.
In the present study, the oral D_7-cholesterol tracer was administered in a stomach-soluble gelatine capsule with a standardized breakfast, instead of being solubilized in an oily or fatty substance. Theoretically, this may have hampered incorporation of the oral tracer into mixed micelles and thereby absorption of the oral D_7-cholesterol tracer, possibly resulting in lower measured FCA.

Methods

We measured plasma D_7-cholesterol enrichment over a period of seven days after administration of the oral D_7-cholesterol tracer in three different ways: i. 50 mg D_7-cholesterol in powder form in a capsule with a standard breakfast, identical to our study (test 0); ii. 50 mg D_7-cholesterol in powder form in a capsule with a high-fat breakfast (test 1); iii. 50 mg D_7-cholesterol dissolved in cacaobutter in a capsule, with the standard breakfast (test 2). This was done in a crossover experiment, with 2-week intervals between the study periods. During each study period, plasma D_7-cholesterol enrichment was measured at T=0, T=9, T=11, T=24, T=48, T=72, T=120 and T=168h after ingestion of the D_7-cholesterol. The study population consisted of eight randomly selected male subjects from the 80 participants of our study, who still met the original in- and exclusion criteria (Supplemental Table 1). The composition of the breakfasts is described in table 2.

Supplemental Table 1. Subjects characteristics (N=8, males)

Age (years)	61.6 ± 8.2
BMI (kg/m2)	28.2 ± 2.2
Systolic blood pressure (mmHg)	150 ± 9
Diastolic blood pressure (mmHg)	88 ± 8
Total cholesterol (mmol/L)	5.75 ± 0.87
LDL-cholesterol (mmol/L)	3.73 ± 0.55
HDL-cholesterol (mmol/L)	1.42 ± 0.27
Triglycerides (mmol/L)	1.25 [0.73 -2.07]
Campesterol (mg/dl)	0.64 ± 0.24
Campesterol/TC (ug/mg)	1.10 ± 0.33

Data are presented as means ± SD. Triglycerides are shown as median [range].

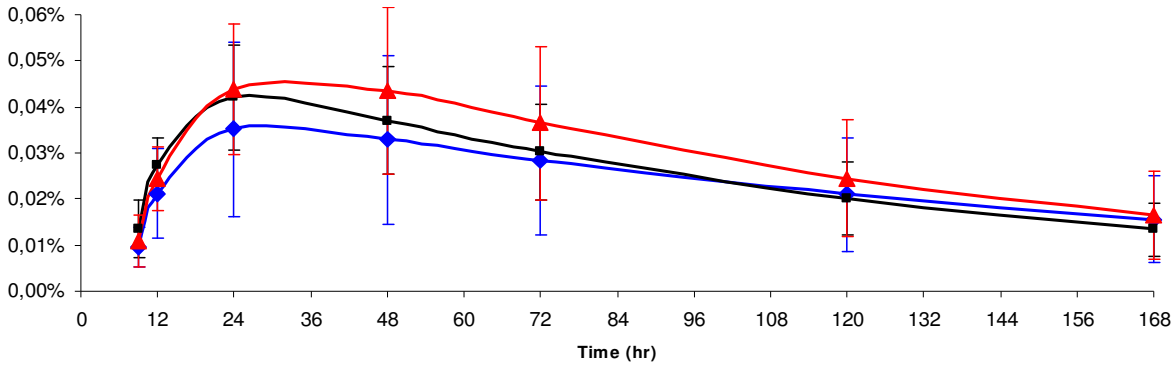
Supplemental Table 2. Composition of breakfasts

	Test 0	Test 1	Test 2
Composition	Standard breakfast*	Standard breakfast + 30 g margarine and one extra slice of bread with marmalade	Standard breakfast + one extra slice of bread with marmalade
Administration of oral D_7-cholesterol	50 mg powder in one capsule	50 mg powder in one capsule	50 mg dissolved in 2385 ± 8,5 mg cacao butter, divided over 4 capsules
Energy (kcal) - Protein (kcal) - Carbohydrate (kcal) - Fat (kcal)	458 100 211 148	769 115 323 332	610 115 323 173
Cholesterol (mg)	29	31	30

*The standard breakfast was identical to the breakfast provided in the main study, consisting of 2 slices of whole wheat bread with 15g of margarine, 40g cheese, 150ml semi-skimmed milk, and coffee with a small slice of gingerbread.

We found no significant difference in the plasma D_7 cholesterol enrichment curves between the three study periods, as determined by testing the AUC on a per subject basis (Supplemental Figure 1). This rules out the option that the observed somewhat lower fractional cholesterol absorption rates can be attributed to the method of administration of the oral D_7 -cholesterol tracer.

Supplemental Figure 1. Plasma D_7 cholesterol enrichment curves



No significant differences between plasma D7-enrichment curves after the original method of administration (rectangles); the high fat breakfast (triangles) and the D_7-cholesterol dissolved in cacaobutter with a normal breakfast (diamonds) at either of the timepoints.